

**Product No. D-0914**  
**2',3'-Dideoxycytidine-(2',3'-<sup>3</sup>H)**  
For Immunoassay

**Lot** 014H8940

Tritiated (<sup>3</sup>H) 2',3'-dideoxycytidine (ddC) for use in immunoassay is supplied ready to use for 100 double antibody radioimmunoassay (RIA) tests when used with Sigma ddC antiserum (Sigma Product No. D-1787).

The ddC-<sup>3</sup>H RIA is a competitive binding immunoassay in which ddC-<sup>3</sup>H and unlabeled ddC (standard or unknown sample) compete for a limited number of combining sites present in the rabbit antiserum to ddC. Separation of the bound and free ddC-<sup>3</sup>H is accomplished using a specific immunoprecipitation reagent containing goat antiserum to rabbit IgG. The ratio of radioactivity bound in the presence of ddC to that bound without ddC is inversely proportional to the concentration of ddC (See sample data).

The ddC-<sup>3</sup>H RIA procedure described in this product insert will allow the determination of as little as 0.3 ng of ddC per assay tube.

**Specific Radiochemical Data**

2',3'-dideoxycytidine-(2',3'-<sup>3</sup>H)

Specific activity	44 Ci/mmol
Total Activity in bottle	0.735 $\mu$ Ci
Activity per ml	0.070 $\mu$ Ci/ml
Volume per bottle	10.5 ml

Diluent: 0.1 M phosphate buffer, pH 7.4 containing 200  $\mu$ g/ml rabbit IgG, 100  $\mu$ g/ml bovine gamma globulins and 0.1% sodium azide (see MSDS)\* as a preservative.

**Sample Data**

Below is an example of a typical antigen addition curve generated using the reagents and methods described in this product insert.

Total Radioactivity (TR) = 12208 DPM  
%Bo/TR = 46.5%  
Non-specific binding (NSB) = 239 DPM  
%NSB/TR = 2.0%

ng/ml	DPM	%B/Bo	Intercepts
0.0	5802	100.0	90% 3.6 ng/ml
3.2	5337	91.7	50% 30.5 ng/ml
8.0	4642	79.2	20% 133.3 ng/ml
20.0	3641	61.2	
50.0	2297	37.0	
125.0	1410	21.1	

**Cross Reactivity**

The specificity of the ddC radioimmunoassay was determined by calculating the ratio of the moles of ddC to moles of ddC analog at the 50% intercept of the respective dose response curves and multiplying the result by 100%.

Analog	%
Cytidine	0.010
2'-Deoxycytidine	0.120
2,3'-Dideoxyuridine	4.105

**Recommended RIA Procedure**

Reagents Required

Item	Sigma Product No.
ddC- <sup>3</sup> H	D-0914
Antiserum to ddC	D-1787
Goat Anti-Rabbit IgG	
Immunoprecipitation Reagent	R-8633
2',3'-Dideoxycytidine	D-5782

**Preparation of Reagents**

ddC-<sup>3</sup>H

Carefully remove the inner seal in the ddC-<sup>3</sup>H bottle and dispose of the inner seal as radioactive waste. Firmly replace bottle cap and store ddC-<sup>3</sup>H at 4°C in the appropriate location. Under proper storage conditions, the ddC-<sup>3</sup>H will be stable for 1 year.

### RIA Assay Buffer

The RIA assay buffer consists of 0.1 M phosphate, pH 7.4 containing 0.1% sodium azide and 0.01% bovine gamma globulins and is available from Sigma as FPIA Dilution Buffer (Sigma Product No. F-3263).

RIA assay buffer may be prepared by dissolving the contents of 1 bottle of Gal-Pac® phosphate buffer (Sigma Product No. 936-4GP) in approximately 3 liters deionized water. Add 3.8 g sodium azide (Sigma Product No. S-2002), 0.38 g bovine gamma globulins (Sigma Product No. G-5009) and stir until all components are completely dissolved. Bring the volume to 3.8 liters with deionized water. Store RIA assay buffer at 4 °C.

### Dideoxycytidine Standards

Standards should be prepared in a matrix equivalent to the unknown samples. The range of the standards is recommended to be 3 - 125 ng/ml for the procedure described below. Dideoxycytidine (D-5782) is readily soluble in RIA assay buffer. Dideoxycytidine standards are stable at 4 °C with a preservative such as 0.1% sodium azide.

### Dideoxycytidine Antiserum

Reconstitute the ddC antiserum with 10 ml RIA assay buffer to obtain working strength antiserum. Store reconstituted antiserum at 4 °C.

### Rabbit IgG Immunoprecipitation Reagent

Rabbit IgG Immunoprecipitation Reagent is supplied as a ready to use mixture of goat antiserum to rabbit IgG in 0.1 M phosphate buffer, pH 7.4, containing 5 mM EDTA, 3.9% polyethylene glycol and 0.01% thimerosal as a preservative. Before use, gently mix by inversion. Store the immunoprecipitation reagent at 4 °C.

### **Procedure**

1. Label 12 x 75 mm test tubes with the appropriate standard or test sample identification.
2. Pipet 100 µl of ddC-<sup>3</sup>H to all test tubes.  
Optional: Add 100 µl ddC-<sup>3</sup>H to total radioactivity tubes (TR) and non-specific binding tubes (NSB).
3. Pipet 100 µl standard or test sample to the appropriate test tube.  
Optional: Add 100 µl standard 0 to the NSB tubes.
4. Pipet 100 µl antiserum to all tubes (except optional TR and NSB tubes).  
Optional: Add 100 µl RIA assay buffer to NSB tubes.
5. Vortex gently to ensure complete mixing and incubate at ambient temperature for 60 minutes.
6. Pipet 1 ml Rabbit IgG Immunoprecipitation Reagent to all tubes (except optional TR tubes). Mix gently.
7. Centrifuge at 2,000xg for 15 minutes at 4 °C.
8. Aspirate the supernatant from each tube (except optional TR tube).
9. Pipet 600 µl 0.1 N HCl to each tube (except optional TR tube) and mix well.  
Optional: Add 500 µl 0.1 N HCl to TR tube.
10. Transfer 500 µl of the contents of each tube into liquid scintillation vials and add liquid scintillation solution.
11. Count each sample for a minimum of 2 minutes, reduce data and calculate results as appropriate.

\*Due to sodium azide content a material safety data sheet (MSDS) for this product has been sent to the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

### **Reference**

Mitsuya, H., and S. Broder, Proc. Natl. Acad. Sci. USA, **83**, 1911 (1986).